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Isolation, fractionation and identification of chemical constituents from the leaves crude extracts of *Mentha piperita* L grown in Sultanate of Oman

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PEER REVIEW

Peer reviewer

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Comments

The present study on biochemical screening of various leaves crude extracts of *M. piperita* provide the valuable brief and scientific information about this plant.
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ABSTRACT

Objective: To analyze and identify the chemical compositions of different organic plants crude extracts of *Mentha piperita* (*M. piperita*) grown in Sultanate of Oman by gas chromatography-mass spectrometry (GC-MS).
Methods: The powder sample was extracted with methanol by using Soxhlet extractor. Methanol crude extracts of *M. piperita* and its derived fractions of hexane, chloroform, ethyl acetate and butanol were prepared.
Results: Qualitative analyses of various organic plant crude extracts of *M. piperita* by using GC-MS showed that majority of these compounds are bioactive.
Conclusions: According to the results of the present study, the plant crude extracts could be used as medicine for the treatment of different diseases. The analysis and identification of the chemical compounds in the plant crude extracts by using GC-MS was the first time.

KEYWORDS
Mentha piperita, Organic crude extracts, Soxhlet extractor, Gas chromatography-mass spectrometry analyses

1. Introduction

Mentha piperita L (*M. piperita*) is one of the most important medicinal plants in folk medicine. This plant is a leafy plant belonging to the family of Lamiaceae. Since the ancient times, different parts of this medicinal plant have been used to cure specific ailments. It is indigenous to Europe. Nowadays it is cultivated throughout all regions of the world[1]. Ancient Greek, Romanium and Egyptian cultures used this plant for cooking purpose as well as preparation of medicine[2,3]. *M. piperita* is a Latin name comes from the Greek Mintha. Various types of mint species are available worldwide such as applemint, water mint, horsemint, pineapple mint, orange mint, pennyroyal and spearmint. The leaves of this plant contain some important

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bioactive compounds. The leaves also contain one toxic compound pennyroyal. This toxic compound can damage the liver[4]. But the essential oil of this plant is rubbed onto the skin to repel insects and prevent them from biting[3]. Recently, there is growing interest among the scientists and researchers for the preparation of new drugs derived from the medicinal plants. At the same time their interest is on stems and leaves of this plant to isolate and identify some bioactive compounds which can be used as a medicine for the treatment of diseases. They belief that medicine derived from stems and leaves are safe and dependable. The isolated drugs from this plant can compare with costly synthetic drugs that have adverse effects[2,3].

The world production of *M. piperita* oil is about 8 000 tons per year[4] due to its medicinal values. The plant contains high concentration of essential oil around 1.2%-1.5%. The essential oil of this plant contains some active ingredients, such as menthol, menthone, and menthyl acetate[1-5]. There are other active ingredients also found in the *M. piperita*, such as flavonoids, polymerized polyphenols, carotenes, tocopherols, saponin, and choline[1-5]. The essential oil is useful for mental fatigue and depression, and also refreshing the spirit, stimulating mental agility and improving concentration. It is widely used in food, cosmetics, medicines[5-7], and it is also used for chemopreventive and antimutagenic purpose[8]. It is also used for symptomatic relief of the common cold[6].

In China, the formulation products from *M. piperita* are used to decrease symptoms of irritable bowel symdrome and decrease digestive symptoms such as dyspepsia and nausea[5-7]. Traditionally, it is used as an analgesic and to treat headache[9]. Commercially, it is used for the production of manufactured products, cooking and medicine for its aromaticity[9]. Recently, this plant is used for the preparation of toothpaste, chewing gum, mouthwash, soaps, sweets, balms or creams and cough medicine[2-5]. The literature search reveals that still no works has been done on the crude extracts of Omani *M. piperita* species. Therefore, the aim of the work was to isolate and investigate the chemical constituents in different organic plant crude extracts from the leaves of locally grown medicinal plants *M. piperita* by using gas chromatography-mass spectrometry (GC-MS).

2. Materials and methods

2.1. Chemicals

All chemicals such as hexane, chloroform, methanol, butanol and ethyl acetate (analytical grade) were purchased from BDH Ltd., UK and the other high performance liquid chromatography grade solvents from Sigma Chemical Co.

2.2. Plant sample

The plant samples of *M. piperita* were collected from Nizwa, Sultanate of Oman. Initially, it was identified by morphological features and database available in the website. The collected plant samples were transported to the laboratory and kept at room temperature until analysis.

2.3. Sample collection

The whole *M. piperita* plants were collected from Nizwa, Sultanate of Oman. The plants were harvested in the month of March, 2012 at 5 PM. The collected plant samples were packed in polyethylene bags and stored at 4 °C until further process.

2.4. Preparation of samples

The separated leave samples were washed with tap water to remove the dust and other foreign materials. The washed *M. piperita* samples were dried under shade for 3 d. Approximately about 100 g of leaves was ground using a grinder for 20 seconds. The air-dried whole leaves were pulverized into powdered form by using heavy duty blander (Jaipan, Super Deluxe, India).

2.5. Preparation of crude extracts

The powder samples (50 g) were extracted with methanol solvent (500 mL) by using Soxhlet extractor for 72 h. After complete extraction, the methanol solvent was evaporated by using rotary evaporator (Yamato, Rotary Evaporator, model-RE 801) under reduced pressure to obtain methanol crude extract (3.59 g). The methanol crude extract from *M. piperita* (3.0 g) was suspended in water (60 mL). Then it was extracted successively with different organic solvents such as hexane, chloroform, ethyl acetate and butanol to obtain hexane (1.09 g), ethyl acetate (0.45 g), chloroform (0.16 g) and butanol (0.38 g) and residual methanol fractions (1.78 g), respectively. All crude extracts were filtered separately through Whatman No. 41 filter paper to remove particles. The particle free crude extract was evaporated completely by using rotary evaporator (Yamato, Rotary Evaporator, model-RE 801) under reduced pressure to obtain dry crude extracts. The residue left in the separatory funnel was re-extracted twice follow the same procedure and filtered. The combined extracts were concentrated and dried by using rotary evaporator under reduced pressure.

2.6. GC-MS analysis

The GC-MS analysis of different organic plant crude extracts from the leaves of *M. piperita* grown in Sultanate of Oman was performed using GC-MS. The analysis done by using a PerkinElmer Clarus 600 GC system was equipped with a fused silica gel column (30 m×0.25mm ID, film thickness 0.25 μm) coupled with a PerkinElmer Clarus 600C MS. The detection of data or spectra was done using an electron ionization system with ionization energy of 70 eV. Inert helium gas (99.999%) was used as a carrier gas at a constant flow rate of ±1 mL/min. Mass transfer line and injector temperatures were at 220 and 290 °C, respectively. The temperature programmed for oven was from

60 °C (hold 2 min) to 270 °C at 4 °C/min, then held isothermal for 20 min and finally raised to 300 °C at 10 °C/min. The crude tested samples were diluted with methanol (1/100, v/v, in methanol). The tested samples were filtered with 0.45 μm Millex membrane filter paper (Millipore, France) to remove any dust particles. One microliter filtered test sample was injected in the split mode. The split ratio was 120:1. The percentage (%) of the crude extracts constituents from the *M. piperita* was expressed as percentage by peak area. The whole process was carried out carefully from the light and heat.

2.7. Identification of chemical constituents

The bioactive compounds were analyzed and identified in different plant crude extracts from the Omani *M. piperita* based on GC retention time on Rtx®-5MS fused silica capillary column. The mass spectra were matched with computer matching with those of standards (NIST 2005 v.2.0 and Wiley Access Pak v.7, 2003 of GC-MS systems). If it is possible by co-injection was matched with authentic compounds[10].

3. Results

The powdered leave samples were extracted with methanol by usual procedure. The methanol was evaporated from the extract using rotary evaporator to obtain semi solid masses. The methanol crude extract was defatted with water and extracted with different solvent with increasing of polarities to obtain hexane, ethyl acetate, chloroform, butanol and residual methanol fractions, respectively.

3.1. Physical properties

The different crude extracts from *M. piperita* leaves was different in colours. The hexane extract was deep brown in colour, ethyl acetate was pale yellow, chloroform extract was deep orange and the butanol extract was deep blackish.

3.2. Chemical composition of different crude extracts

The hexane crude extract from *M. piperita* was analyzed by using GC-MS system with fused silica gel column (Rtx®-5MS) and 18 different organic compounds were found, representing 1.09% of the total extract. The separated identified chemical compounds in hexane crude extract are listed in Table 1 according to their retention time on Rtx®-5MS capillary column. The major chemical compounds identified in the hexane extract from Omani *M. piperita* (shown in Figure 1 and Table 1) included eucalyptol (1.697%), 3-octanol (0.592%), borneol (0.894%), dihydrocarveol (3.512%), pulegone (3.562%), carvone (8.498%), caryophyllene (5.002%), β-cuvebene (4.658%), hexadecylene oxide (3.102%), N-hexadecylene oxide (7.709%), phytol (1.06%), α-linolenic acid (26.785%), 2-monopalmitin (2.175%), α-amyrin (4.801%), squalene (0.986%) and vitamin E (5.577%).

Table 1
Chemical composition of hexane crude extract of *M. piperita*.

Name of compounds	Retention time (min)	Leave (%)
3-Octanol	7.246	0.592
Eucalyptol	8.471	1.697
3-Octanol acetate	11.823	0.333
Borneol	13.538	0.894
Dihydrocarveol	14.684	3.512
Pulegone	16.520	3.562
Carvone	16.705	8.498
Caryophyllene	23.903	5.002
β-Cuvebene	26.359	16.878
Hexadecylene oxide	39.189	3.102
N-Hexadecylene oxide	43.156	7.709
Phytol	47.738	4.658
α-Linolenic acid	48.523	26.785
2-Monopalmitin	58.387	2.175
α-Amyrin	62.914	4.801
Squalene	65.956	0.986
Vitamin E	72.483	5.577

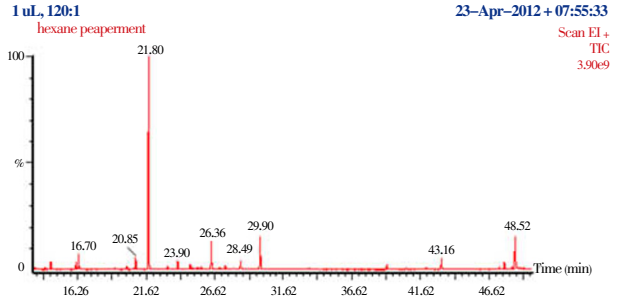

Figure 1. A typical gas chromatogram of the chemical constituents of hexane extract.

The ethyl acetate crude extract was analyzed by using GC-MS, and it was found to contain a total of 7 different organic bioactive compounds using the same capillary column and conditions, representing 0.45% of the total extract. The major chemical constituents were found in the ethyl acetate extract (Figure 2 and Table 2), including benzoic acid (1.896%), hydrochlorobutanolic acid (2.964%), caffeic acid (31.963%), benzamide acetate (9.073%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (8.965%), phytol (4.037%) and 9,12,15-octadecatrienal (9.077%).

Table 2
Chemical composition of ethyl acetate crude extract of *M. piperita*.

Name of compounds	Retention time (min)	Leave (%)
Benzoic acid	16.71	1.896
Hydrochlorbetanolic acid	20.51	2.964
Caffeic acid	21.78	31.963
Benzamide acetate	24.81	9.073
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	39.20	8.965
Phytol	47.73	4.037
9,12,15-Octadecatrienal	48.47	9.077

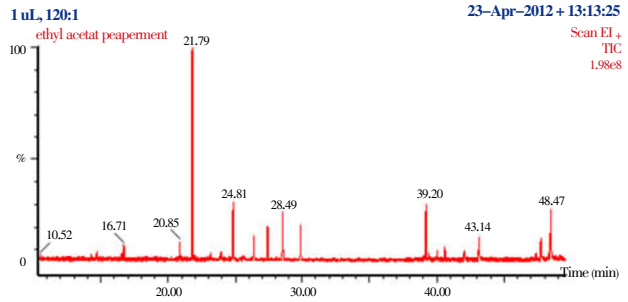

Figure 2. A typical gas chromatogram of the chemical constituents of ethyl acetate extract.

The chloroform extract from the plant of *M. piperita* was analyzed by using GC-MS, and it was detected to contain 6 different organic compounds using the same capillary column and conditions, representing 0.16% of the total extract. The major chemical constituents that were found in the chloroform extract (Figure 3 and Table 3) include gluconic acid (9.527%), 2-p-tolylpropene (3.783%), cis-verbenone (3.786%), L-perillaldehyde (59.266%), phytol (13.412%) and α-linolenic acid (10.223%).

Table 3

Chemical composition of chloroform crude extract of *M. piperita*.

Name of compounds	Retention time (min)	Leave (%)
Gluconic acid	8.201	9.527
2-p-Tolylpropene	10.527	3.783
Cis-Verbenone	20.727	3.786
L-perillaldehyde	21.782	59.266
Phytol	39.190	13.412
α-Linolenic acid	48.454	10.223

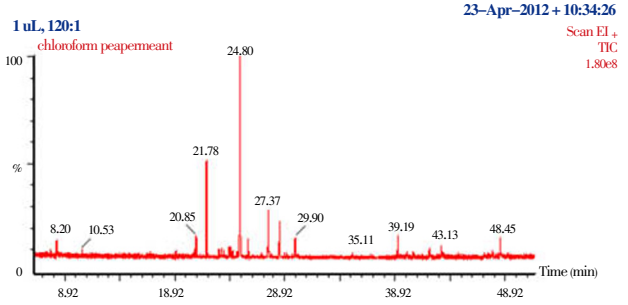

Figure 3. A typical gas chromatogram of the chemical constituents of chloroform extract.

The butanol extract from the plant of *M. piperita* was analyzed by using GC-MS with the same condition as above, and the analyis led to the identification of 7 different organic compounds, representing 0.38% of the total extract from samples. The major chemical constituents in the butanol crude extract (Figure 4 and Table 4) were 2-p-tolylpropene (19.282%), cis-verbenone (17.966%), 2,6-cresotaldehyde (24.690%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (14.060%), n-hexadecoic acid (5.098%) and phytol (2.405%).

Table 4

Chemical composition of butanol crude extract of *M. piperita*.

Name of compounds	Retention time (min)	Leave (%)
2-p-Tolylpropene	10.53	19.282
cis-Verbenone	20.72	17.966
2,6-Cresotaldehyde	24.81	24.690
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	39.18	14.060
n-Hexadecoic acid	43.13	5.2976
Phytol	47.74	2.4054

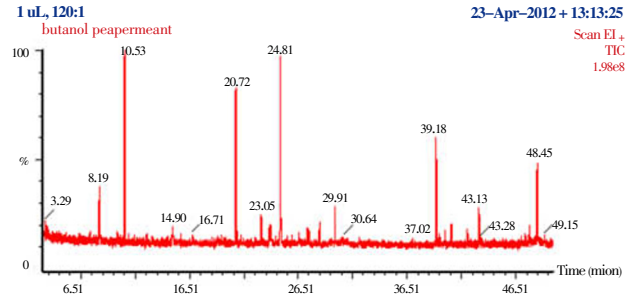

Figure 4. A typical gas chromatogram of the chemical constituents of butanol extract.

Finally the residual methanol crude extract was analyzed by using GC-MS, and led to the identification of 8 different organic compounds, representing 1.78% of the total extract from plant samples. The major chemical constituents in the methanol crude extract (Figure 5 and Table 5) were p-isopropenyl toluene (16.153%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydrox (3.026%), (+)-carvone (4.655%), germacrene D (5.801%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (7.047%), palmitic acid (5.328%), 9,12,15-octadecatrien-1-ol (12.099%) and β-sitosterol (45.886%).

Table 5

Chemical composition of methanol crude extract of *M. piperita*.

Name of compounds	Retention time (min)	Leave (%)
P-isopropenyl toluene	10.527	16.153
4H-pyran-4-one, 2,3-dihydro-3,5-dihydrox	12.538	3.026
(+)-Carvone	16.715	4.655
Germacrene D	26.359	5.801
3,7,11,15-tetramethyl-2-hexadecen-1-ol	39.195	7.047
Palmitic acid	43.131	5.328
9,12,15-octadecatrien-1-ol	48.474	12.099
β-sitosterol	56.147	45.886

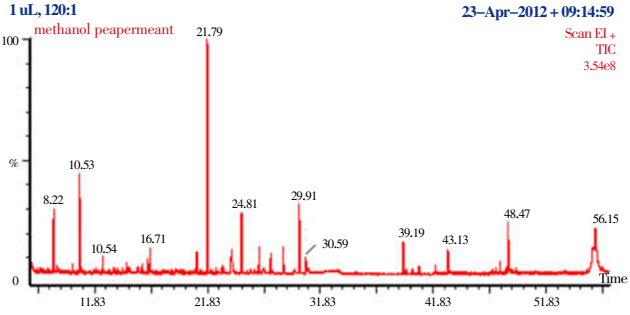

Figure 5. A typical gas chromatogram of the chemical constituents of methanol crude extract.

4. Discussion

All the plant crude extracts were found to obtain some biologically active compounds at very high concentration. The high concentration of major compounds may be considered to be a part of plants defense systems.

The prepared suitable crude extracts was obtained from the leaves of *M. piperita* for bioactive chemical compounds, which can be chosen on the basis of above mentioned GC-MS analysis. All plant crude extracts contained some biologically active compounds at very high concentration. The high concentration of major compounds may be considered to be a part of plants defense systems. They have been included in a large group of protective molecules found in this plant named 'phytoanticipins' or 'phytoprotectants'[11,12].

Therefore, the separation and identification of various crude extracts from the leaves of *M. piperita* by GC-MS were needed. The identified chemical compounds might have some important ecological significance. The majority of chemical constituents present in the crude extracts of Omani *M. piperita* have not been previously reported.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

M. piperita is one of the most important medicinal plants in folk medicine. This plant is a leafy plant belonging to the family of Lamiaceae. Locally it is known as "pippermint" and their dried whole parts are used in folk medicine. According to the literature search, there is no work has been done on Omani *M. piperita* L by the researcher.

Research frontiers

The aim of this study is to prepare various crude extracts using different polarities of solvent and to qualitatively evaluate their chemical constituents by GC-MS.

Related reports

The literature search reveals that there is no work has been done on Omani *M. piperita* species by the researcher. Nevertheless, the other parameters of this plant has been investigated by other researchers.

Innovations and breakthroughs

Although the experimental work done by the author is routine work, it gives the new information and data to the scientific community.

Applications

This plant is used worldwide as a medicine. According to the paper, there are so many bioactive compounds that can be used to prepare medicine.

Peer review

The present study on biochemical screening of various leaves crude extracts of *M. piperita* provides the valuable brief and scientific information about this plant.

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